REMARKS

INTRODUCTORY COMMENTS:

Claims 26-37 were examined in the Office Action under reply, remaining claims 1-25 and 38-44 having been withdrawn from consideration as a result of restriction. The examined claims stand rejected as follows:

(1) under 35 U.S.C. §102(b) as anticipated by Bernard et al. (claims 26, 27 and 33);

(2) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Lee et al. (claims 28 and 29);

(3) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Chick et al. (claims 30-32); and

(4) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Dykens et al. (claims 34-37).

The rejections are addressed in part by the amendment to claim 26 and are otherwise traversed for reasons that will be discussed in detail below.

THE REQUIREMENT FOR RESTRICTION:

Restriction has been required to one of the following subject matter groupings:

I. Claims 1-25, drawn to a method for detecting the proximity of two molecular segments using fluorescein-cyanine 5 as a dye pair;

II. Claims 26-37, drawn to a composition having a binding pair comprised of a first member attached to fluorescein and a second member attached to cyanine 5;

III. Claims 38-43, drawn to a compound having a first segment covalently bound to fluorescein and a second segment covalently bound to cyanine 5; and

IV. Claim 44, drawn to a fluorescence resonance energy transfer ("FRET")-based assay using fluorescein and cyanine 5 as a dye pair. Applicants provisionally elected the claims of Group II in the telephone conference conducted with the Examiner on October 9, 2001, with traverse. By way of this response, applicants now affirm the provisional election of the claims of Group (II), and traverse the restriction requirement on the following grounds.

In making the restriction requirement, the Examiner alleged that the claims of Groups I, II, III and IV are unrelated and independent inventions. Specifically, the Examiner has stated that the claim groups are "unrelated" on the ground that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects, citing the MPEP at sections 806.04 and 808.01. In the Examiner's opinion, the different "inventions" claimed have different modes of operation, e.g., the "feature of a method of detecting the proximity of Group I" is not required by the claims of the other groups. Similarly, the Examiner argued that the "feature of a composition of a binding pair of Group II" is not required by the claims of the other groups.

Applicants respectfully disagree, and submit that the claims can easily be examined as is without any undue burden on the PTO, since they all recite cyanine 5 and fluorescein as a dye pair. Any search conducted on one claim group would be equally applicable to the other claim groups. On this point, applicants refer the Examiner to Section 803 of the M.P.E.P., where it is stated that "[i]f the search and examination of an entire application can be made without serious burden, the examiner **must** examine it on the merits...." This is true even though an application may include claims to distinct or independent inventions.

Further, applicants disagree with the Examiner's assertion that the claimed inventions are not disclosed as capable of use together and have different modes of operation, functions, or effects. For example, claim 44 (Group IV) is directed to an improved FRET-based assay wherein the improvement is based upon use of fluorescein and cyanine 5 as a dye pair, and claims 26-37 (Group II) are directed to a composition having a binding pair comprised of a first member attached to fluorescein and a second member attached to cyanine 5. Restriction here is inappropriate since both can be used together, i.e., the claimed compositions are clearly intended to be used in the claimed FRET-based assay. Similarly, the composition having a binding pair comprised of a first member attached to fluorescein and a second member attached to cyanine 5, as claimed in claims 26-37 (Group II), is clearly intended to be used in the method for detecting the proximity of two molecular segments using fluorescein-cyanine 5 as a dye pair, as claimed in claims 1-25 (Group I).

In addition, the compound having a first segment covalently bound to fluorescein and a second segment covalently bound to cyanine 5, as claimed in claims 38-43 (Group III), functions

in the same way and brings about the same result as the composition of Group II, having a binding pair comprised of a first member attached to fluorescein and a second member attached to cyanine 5, as claimed in claims 26-37 (Group II).

Furthermore, the compound having a first segment covalently bound to fluorescein and a second segment covalently bound to cyanine 5, as recited in claims 38-43 (Group III), is clearly capable of use in the method for detecting the proximity of two molecular segments using fluorescein-cyanine 5 as a dye pair, as recited in claims 1-25 (Group I).

Applicants thus respectfully submit that as the various claim groups are indeed disclosed as capable of use together, and have the same modes of operation, the same functions, and the same effects. Therefore, applicants respectfully request that the restriction requirement be reconsidered and withdrawn.

THE ABOVE AMENDMENTS:

Independent claim 26 has been amended to recite that the binding pairs are selected from the group consisting of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor, enzyme-substrate and fragments or analogs thereof. This amendment is supported in the application on page 10, last paragraph, and in the claims as originally filed.

THE REJECTION UNDER 35 U.S.C. § 102(b):

Claims 26, 27 and 33 were rejected under 35 U.S.C. §102(b) as being anticipated by Bernard et al. (1998), *Anal. Biochem.* 255:101-107 ("Bernard"). With reference to claim 26, the Examiner stated that Bernard discloses a method of using fluorescein and cyanine 5 as a dye pair for fluorescence resonance energy transfer in the examination of hybridization probes. With respect to claim 33, the Examiner stated that the method was used to examine the fluorescence energy transfer between a cyanine 5-labeled PCR strand and a complementary fluorescein probe. With respect to claim 27, the Examiner stated that Bernard further discloses that cyanine 5 and fluorescein were covalently attached to the probe by determining the ratio of oligonucleotide concentration to fluorophore concentration, and that if every oligonucleotide strand is fluorescently labeled, and if no fluorophore were present, the ratio of oligonucleotide

concentration to fluorophore concentration should be 1.0.

Applicants respectfully traverse this rejection. Bernard teaches that cyanine 5 and fluorescein may be used for fluorescence energy transfer, but only in the context of oligonucleotide hybridization. Further, Bernard specifically makes note that the fluorescence energy transfer was only observed when the chromophores were separated by a 3-bp gap. Bernard states that the efficiency of energy transfer between donor and acceptor fluorophores in DNA varies according to the relative positions on the DNA helix, with a sigmoidal energy transfer efficiency curve [as a function of position along the sequence] being observed due to the helical geometry of the DNA. Bernard specifically teaches that the energy transfer between fluorophores on complementary strands is maximal when there are only 3 or 4 intervening base pairs, and that less fluorescence was developed during amplification when the fluorophores are separated by 10 or 15 bases, or even when there were no intervening base pairs, such that the fluorophores were disposed on opposite sides of the helix. See Figure 1 legend and page 106, first full paragraph.

It is well known in the art that base stacking occurs in helical double stranded DNA, and that the separation between adjacent bases is 3.4Å. (Stryer, *Biochemistry,* 3rd Ed., page 76 (New York: W.H. Freeman and Co., N.Y., 1988)). Therefore, it is well known in the art that fluorophores attached to oligonucleotides having a separation of 3 bp would be separated by a distance of no more than about 10Å. In other words, Bernard teaches that fluorescence energy transfer can occur between cyanine 5 and fluorescein only under very specific and restricted experimental conditions, where virtually no separation exists between the fluorophores.

An examination of the equation governing energy transfer between fluorophores (See Schobel et al. (1999), *Bioconjugate Chem.* 10:1108) shows that there are numerous factors that control energy transfer efficiencies, such as distance, orientation factors and the overlap integral between the donor emission spectrum and the acceptor excitation spectrum. Where there is very little if any overlap in the donor emission and the acceptor excitation spectra, in order for FRET to occur, the fluorophores must be very close and oriented for maximum efficiency in order for energy transfer to be observable.

Applicants respectfully submit that the claims as amended are patentable over Bernard. "A claim is anticipated only if each and every element as set forth in the claim is found, either

expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Claim 26 recites:

> 26. (Amended) A composition comprising a first member of a binding pair directly or indirectly attached to fluorescein and a second member of the binding pair directly or indirectly attached to cyanine 5, wherein the first and second members of the binding pair are associated so that the fluorescein and cyanine 5 are in fluorescence resonance energy transfer proximity to each other, wherein said binding pairs are selected from the group consisting of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor, enzyme-substrate and fragments or analogs thereof.

Thus, claim 26, and claims 27 and 33, which depend from claim 26, are not anticipated by Bernard because Bernard does not teach each and every element as set forth in claim 26. There is no teaching or suggestion in Bernard that use of fluorescein and cyanine 5 in FRET in situations outside oligonucleotide hybridization would be successful. Further, Bernard teaches that fluorescein did not successfully transfer energy to cyanine 5 unless the fluorophores were in very close proximity. Therefore, Bernard does not anticipate claims 26, 27 or 33 as presently claimed.

Further, applicants respectfully submit that the present claims would not be obvious over the disclosure of Bernard. Given the uncertainty in the separation between binding pairs in the context of antigen-antibody, receptor-ligand and enzyme-substrate interactions, for example, one skilled in the art would not have a reasonable expectation of success in applying FRET using fluorescein-cy5 as a dye pair outside the context of oligonucleotide interactions.

Therefore, applicants respectfully submit that the rejection of claims 26, 27 and 33 under 35 U.S.C. §102(b) have been overcome and request that the rejections be withdrawn.

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 28 AND 29 AS OBVIOUS OVER BERNARD IN VIEW OF LEE:

Claims 28 and 29 were rejected as obvious over Bernard in view of Lee et al. (1995), *Anal. Biochem.* 227:295-301 ("Lee"). The Examiner has conceded that Bernard does not explicitly disclose an indirect attachment of fluorescein and Cy5, but has alleged that Lee

discloses that the fluorophore moieties are attached via a linker in order to confirm the absence of fluorescence quenching due to the probe-DNA interactions when the probe is introduced to the DNA via a longer linker arm. The Examiner has stated that it would have been obvious to provide the composition of Bernard with a linker as taught by Lee in order to confirm the absence of fluorescence quenching due to the probe-DNA interactions when the probe is introduced to the DNA via a longer linker arm.

Applicants respectfully traverse the rejection. Lee teaches that fluorophore fluorescence may be quenched due to the existence of fluorophore-DNA interactions. The linker between the fluorophore and the DNA, discussed by Lee, was provided to ensure sufficient distance between the probe and the DNA molecule as an experimental control so that fluorescence quenching by DNA could be distinguished from fluorescence resonance energy transfer. Lee discusses the observed donor fluorescence quenching, and concludes that the quenching was attributable to energy transfer, rather than probe-DNA quenching. See Lee at page 297, right col., lines 7-10.

While Lee may teach that a fluorophore can be connected to a linker in order to confirm the absence of fluorescence quenching due to probe-DNA interactions, claims 28 and 29 are not concerned with confirming the absence of fluorescence quenching. In contrast to Lee, the presently claimed invention is directed to fluorescence resonance energy transfer by observing the acceptor fluorophore's fluorescent emission, and is not concerned with DNA quenching of fluorescence. Claim 28 recites that the first member of the binding pair is indirectly attached to fluorescein and the second member of the binding pair is indirectly attached to cyanine 5, and the indirect attachment is effected through one or more linking moieties. Claim 29 recites that at least one member of the binding pair is indirectly attached to either fluorescein or cyanine 5, and the indirect attachment is effected through one or more linking moieties. Further, the claims are directed to a composition comprising specific binding pairs, as claimed in amended independent claim 26. Therefore there is no teaching or suggestion in Lee that in combination with the teachings of Bernard gives rise to the present claims 28 and 29. Therefore, for at least this reason, the combination of the disclosures of Lee and Bernard does not render the present claims unpatentable.

Further, in claims 28 and 29, indirect attachment of the fluorophore to the binding pairs is not obvious over Bernard in view of Lee because Bernard teaches that FRET only occurs when

fluorescein and cyanine 5 are separated by no more than about 3-4 base pairs. The use of a linker as disclosed by Lee would not be suggested by the pending claims because Bernard specifically teaches that these two probes must be in close proximity in order for FRET to occur. One skilled in the art would not read Lee in combination with Bernard and conclude that fluorescein and cyanine 5 would be efficient for FRET if separated by greater distances, as for example, if the fluorophores were connected to the members of binding pairs by linkers, thereby separating the fluorophores further. Given the disclosures of Bernard and Lee, one skilled in the art would not even consider it advantageous to try linking fluorescein and cyanine 5 to oligonucleotides, out of concern for excess separation between the fluorophores. In other words, Bernard teaches away from the use of linkers with fluorescein and cyanine 5. Therefore, for this reason as well, the combination of the disclosures of Lee and Bernard does not render the present claims unpatentable.

Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection over Bernard in view of Lee is respectfully requested.

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 30-32 AS OBVIOUS OVER BERNARD IN VIEW OF CHICK:

Claims 30-32 were rejected as obvious over Bernard in view of Chick et al. (U.S. Patent No. 6,040,194)("Chick"). The Examiner has cited Bernard as above, as disclosing a method of using fluorescein and cyanine 5 as a dye-pair for FRET. The Examiner has conceded that Bernard fails to disclose using this composition to examine antibody-antigen, receptor-ligand or enzyme-substrate interactions; however, the Examiner has stated that it would have been obvious to one having ordinary skill in the art at the time the invention was made to provide the composition of Bernard with different combinations of binding pairs as taught by Chick in order to examine the binding ability of either the antigen-antibody, receptor-ligand or enzyme-substrate in a competitive assay.

Applicants respectfully traverse this ground of rejection. As discussed above, Bernard specifically teaches that the energy transfer between fluorophores on complementary strands is maximal when there are only 3 or 4 intervening base pairs, and that less fluorescence was developed during amplification when the fluorophores were separated by 10 or 15 bases, or even

when there were no intervening base pairs, such that the fluorophores were on opposite sides of the helix. In other words, Bernard teaches that fluorescence energy transfer can occur between cyanine 5 and fluorescein only under very specific and restricted experimental conditions, with very little separation between the fluorophores.

With regard to Chick, this reference specifically teaches that the donor and acceptor have overlapping excited state energy levels and states that "[a]ny appropriately selected donor-acceptor pair can be used, provided that the emission of the donor overlaps with the excitation spectra of the acceptor and both members can absorb light energy at one wavelength and emit light energy of a different wavelength." See col. 3, lines 9-13, col. 6, lines 19-21. Chick further states that "[t]he area of overlap between the donor emission and the acceptor absorbance spectra (which is the overlap integral) is of importance," referring to Figure 1 where the overlap integral between the donor emission spectrum and the acceptor excitation spectrum is graphically illustrated. Chick goes on to discuss the theory of FRET and the efficiency of the energy transfer, including the importance of the overlap integral. See col. 8, lines 4-10, and col. 8, line 63- col. 9, line 8. Thus, Chick teaches away from the use of fluorescein with cyanine 5, because these fluorophores do not have overlapping excited state energy levels, regardless of the observations of Bernard. According to the teaching of Chick, one skilled in the art would have no reasonable expectation of success that the combination of fluorescein and cyanine 5 would exhibit fluorescence energy transfer at useful efficiencies, especially in an experimental setting where the orientation and distance factors between the donor and acceptor fluorophores could not be predicted or controlled, such as antigen-antibody or enzyme-inhibitor contexts as claimed, in contrast to the oligonucleotide studies performed by Bernard.

Accordingly, applicants respectfully submit that claims 30-32 are nonobvious over Bernard in view of Chick, and thus request withdrawal of the rejection.

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 34-47 AS OBVIOUS OVER BERNARD IN VIEW OF DYKENS:

Claims 34-37 were rejected as unpatentable over Bernard in view of Dykens et al. (U.S. Patent No. 6,280,981)("Dykens"). The Examiner cited Bernard as before, with Dykens cited as teaching that the efficiency of the resonance energy transfer is dictated largely by the proximity

of the donor and acceptor. The Examiner has further alleged that Dykens discloses the claimed proximity distances.

Applicants respectfully traverse the rejection. Dykens specifically states at col. 15, lines 41-43, that energy transfer between the fluorophores occurs when "the emission spectrum of the donor overlaps the absorption spectrum of the acceptor." Thus, Dykens, like Chick described above, teaches away from the use of the claimed dye pair for FRET.

In addition, as while Dykens may discuss the distance dependence of FRET in general, there is no teaching or suggestion to use the composition as claimed in pending claim 26, from which claims 34-37 depend, which recites binding pairs selected from the group consisting of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor, enzyme-substrate and fragments or analogs thereof. Therefore, the combination of Dykens and Bernard does not render the present claims unpatentable.

Applicants accordingly requests reconsideration and withdrawal of the rejection.

INFORMATION DISCLOSURE STATEMENT:

In the Office Action, the Examiner stated that copies of the references and PTO-1449 forms submitted with the Information Disclosure Statement dated July 18, 2001 were not in the PTO file. Applicants herewith enclose additional copies of the references previously submitted, along with copies of the Information Disclosure Statement and PTO-1449 forms. Also included is a copy of the stamped postcard indicating receipt by the USPTO on July 23, 2001.

Applicants have also enclosed a Supplemental Information Disclosure Statement, including a Form PTO-1449 and a copy of the cited reference, Wittwer et al. (1997), *Biotechniques* 22(1):130-138, referenced in Bernard. Applicants note that Wittwer also relates to the use of fluorescein and cyanine 5 as dye pairs for FRET in the context of DNA hybridization technologies, and demonstrates the use of the fluorescein and cyanine 5 as a dye pair on adjacent hybridized probes in which the fluorophores were positioned only one base pair apart. See pages 131 and 134. Wittwer also notes that the spectral overlap between fluorescein and cyanine 5 is small (see page 137). Therefore, applicants respectfully submit that Wittwer is consistent with Bernard and teaches that fluorescein and cyanine 5 can be used as a FRET dye pair in the context

of hybridized oligonucleotides wherein the fluorophore separation must be controlled so as to provide very close proximity between the probes (only one base pair separation). Therefore, Wittwer teaches away from any application where the probes would be separated by a distance greater than about 1 base pair separation on hybridized oligonucleotides.

Applicants respectfully request that the Examiner review the enclosed references and make them of record in the application file.

CONCLUSION

If the Examiner has any questions concerning this communication, or would like to discuss the application, the art, or other pertinent matters, he is welcome to contact the undersigned attorney at 650-330-0900.

Respectfully submitted,

Date: 1/25/02 By: Dianne E. Reed

Dianne E. Reed
Registration No. 31,292

REED & ASSOCIATES
800 Menlo Avenue, Suite 210
Menlo Park, California 94025
(650) 330-0900 Telephone
(650) 330-0980 Facsimile

F:\Document\0300\0016\Amendment and Response to Restriction Requirement.

APPENDIX A

AMENDMENTS

Please amend claim 26 as follows:

26. (Amended) A composition comprising a first member of a binding pair directly or indirectly attached to fluorescein and a second member of the binding pair directly or indirectly attached to cyanine 5, wherein the first and second members of the binding pair are associated so that the fluorescein and cyanine 5 are in fluorescence resonance energy transfer proximity to each other, wherein said binding pairs are selected from the group consisting of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor, enzyme-substrate and fragments or analogs thereof.